GENOME ANNOUNCEMENT

Complete Genome Sequence of *Beijerinckia indica* subsp. *indica*

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Abstract

***Beijerinckia indica* subsp. *indica* is an aerobic, acidophilic, N_2-fixing soil bacterium known for its abundant production of exoheteropolysaccharide. It is a chemoorganotroph that is phylogenetically closely related to facultative and obligate methanotrophs of the genera *Methylocella* and *Methylocapsa*. Here we report the full genome sequence of this bacterium.**

Beijerinckia indica subsp. *indica* ATCC 9039 is the type strain of the genus *Beijerinckia* (Starkey and De, 1939), a member of the *Rhizobiales* order of the *Alphaproteobacteria*. *Beijerinckia* spp. are commonly found as free-living bacteria in acidic soils, and also in plant rhizosphere and phyllosphere environments (Kennedy, 2005). Research on *Beijerinckia* has suffered from chronic taxonomic confusion, with some strains of *Sphingomonas* and *Azotobacter* being misidentified in the literature: e.g. a "*Beijerinckia"* reported to degrade PAH has been reclassified (Gibson, 1999). However, some *Beijerinckia* spp. have received research attention due to their potential plant-growth-promoting properties (Thuler 2003), and for their production of heteropolysaccharides with potential biotechnological uses (Scampanari 2000).

Genomic DNA from *Beijerinckia indica* subsp. *indica* was used to create 3-Kb, 8-Kb, and 40-Kb DNA libraries. Sequencing, assembly, and automated annotation was performed at the Joint Genome Institute using standard procedures (U.S. Department of Energy; http://www.jgi.doe.gov/sequencing/strategy.html). The total number of paired-end shotgun Sanger reads in the assembly was 33870. In addition, 454 sequence data

were included into the final assembly: large Newbler contigs were chopped into 4975 overlapping fragments of 1000-bp and entered into the assembly as pseudo-reads.

The genome of *B. indica* subsp. *indica* was 4,170,153 bp. In addition, two plasmids of 181,736 and 66,727 bp were present. There are a total of 3982 ORFs predicted using Glimmer, of which 3784 are predicted protein-coding genes and 2695 (70%) have been assigned a predicted function based on BLASTP searches against the KEGG (Kyoto Encyclopedia of Genes and Genomes). There are 134 pseudogenes, 10 rRNA genes and 52 tRNAs. The GC content is 57.0%.

The genome lacks phosphofructokinase, the key enzyme of the Embden-Meyerhof pathway. Instead, it uses the Entner-Doudoroff or pentose phosphate pathways to catabolize sugars, which is typical of free-living *Rhizobiales*. The majority of the genes involved in N_2-fixation are clustered in two genomic islands (10 Kb and 51 Kb), with the notable absence of the *nifS* gene encoding cysteine desulphurase.

Beijerinckia indica is a metabolically versatile bacterium capable of growth on a variety of organic acids, sugars, and alcohols (Kennedy 2005). In contrast, its close phylogenetic cousins *Methylocella* and *Methylocapsa* are highly specialized methanotrophs capable of growth on very few substrates (Dedysh 2005). However, the genome size of *Beijerinckia indica* versus *Methylocella silvestris* (4.17 versus 4.30 Mbp) and the predicted protein-encoding genes (3788 versus 3917) are remarkably similar. A BLAST analysis indicated that the 57% of genes in the genome of *B. indica* have homologues in *M. silvestris* (stringency threshold expectation value (E) of 1e-50). Many key pathways of one-carbon metabolism (such as the serine, ribulose monophosphate, and tetrahydromethanopterin pathways of formaldehyde metabolism) present in *M.*

silvestris are absent in *B. indica*, which confirm experiments showing that the organism is incapable of growth on one-carbon substrates (Dedysh et al 2005). However, an operon encoding a putative propane monooxygenase homologous to soluble propane/methane monooxygenases of *Methylocella silvestris* BL2 was identified. More in-depth comparison of these genomes will help elucidate what defines their very distinct lifestyles.

Nucleotide sequence accession number. The genome sequence and annotation were deposited in Genbank with accession number CP001016.

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